

GAS-LIQUID CHROMATOGRAPHY OF VALERIAN  
SESQUITERPENOIDS

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We have already studied the gas-liquid chromatography of opium alkaloids<sup>1</sup>, plant glycosides<sup>2</sup>, flavonoids<sup>3</sup>, lichen substances<sup>4</sup>, anthraquinones<sup>5</sup>, and coumarins<sup>6</sup>.

NIGAM AND LEVI<sup>7</sup> and BAPAT *et al.*<sup>8</sup> have recently reported on the gas chromatography of sesquiterpene hydrocarbons, but not that of valerian sesquiterpenoids. We have now attempted to make an effective separation of valerian sesquiterpenoids, which will be useful from the chemotaxonomical standpoint.

The present paper describes the results of gas-liquid chromatography, using two kinds of packed columns, of fifteen standard pure samples of valerian sesquiterpenoids and five kinds of Japanese valerian oils.

The relationships between the retention volumes and the chemical structures, and the properties and the contents of sesquiterpenoids in valerian oils are discussed.

## EXPERIMENTAL

*Materials*

All standard samples of valerian sesquiterpenoids used were donated by Prof. T. TAKEMOTO.

Five kinds of valerian plants from different sources were collected in winter, and dried. Two to three grams of dried roots were extracted with methylene chloride-ether solution (1:1, v/v) at room temperature for 7 days. The extracts, after evaporation,

TABLE I

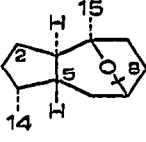
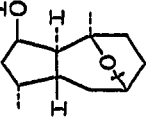
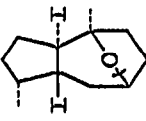
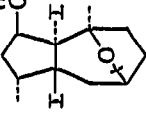
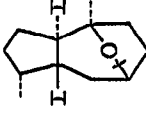
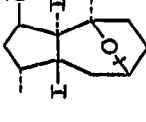
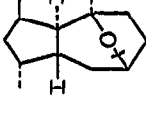
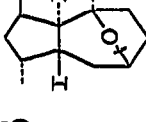
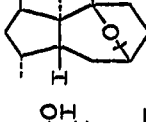
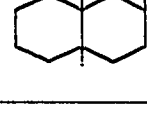
## COLLECTION DATES AND OIL CONTENTS OF VALERIAN PLANTS

A = *Valeriana officinalis* var. *latifolia* (Japanese name, Ka-no-ko-so), grown wild in Kumamoto-ken; B = the same, cultivated at Toyama University; C = the same, cultivated at the Experimental Farm for the Cultivation of Medicinal Plants, the National Hygienic Laboratory; D = the same, cultivated in Tokyo Metropolitan Botanical Gardens; E = *Valeriana flaccidissima*, grown wild in Kanagawa-ken.

Sample	Collection date	Valerian oil content (%)
A	Jan. 16, 1965	2.32
B	Jan. 16, 1965	2.25
C	Jan. 16, 1965	4.27
D	Jan. 18, 1965	2.50
E	Jan. 14, 1965	1.07

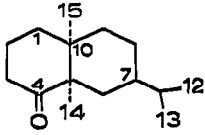
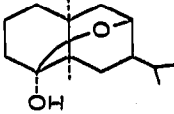
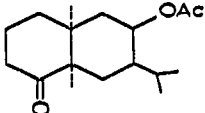
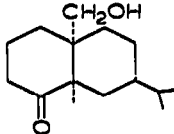
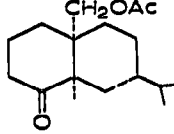
TABLE II

RETENTION TIMES AND RETENTION VOLUMES OF VALERIAN SESQUITERPENOIDS

No.	Compound	Structure	1.5% SE-30 on Chromosorb W		12% DEGS on Gas Chrom P	
			$t_R$ (min)	$V_R$ (ml)	$t_R$ (min)	$V_R$ (ml)
1	Kessane		5.8	351.5	1.5	102.0
2	$\alpha$ -Kessyl alcohol		10.7	648.4	7.2	489.6
3	Kessanol		12.5	757.5	12.4	843.2
4	Kessyl acetate		17.0	1030.2	7.4	503.2
5	Kessanyl acetate		17.8	1078.7	7.0	476.0
6	Kessoglycol		27.0	1636.2	74.1	5038.8
7	8-Acetoxykessan-2-ol*		35.5	2151.3	40.3	2740.4
8	2-Acetoxykessan-8-ol		40.0	2424.0	73.3	4984.4
9	Kessoglycol diacetate		53.7	3254.2	38.2	2597.6
10	Maaliol		6.6	400.0	3.2	217.6

(continued on p. 343)

TABLE II (continued)

No.	Compound	Structure	1.5% SE-30 on Chromosorb W		12% DEGS on Gas Chrom. P	
			$t_R$ (min)	$V_R$ (ml)	$t_R$ (min)	$V_R$ (ml)
11	Valeranone		9.8	593.9	4.8	326.4
12	Cryptofauronol		10.3	624.2	9.4	639.2
13	Fauronyl acetate		27.8	1684.7	23.8	1618.4
14	Kanokonol		28.3	1715.0	65.5	4454.0
15	Kanokonyl acetate		42.3	2563.4	36.7	2495.6

Conditions:

$N_2$ flow rate	60.6 ml/min	68.0 ml/min
Column temp.	150°	180°
Flash heater temp.	250°	250°
Detector temp.	200°	200°

\* Chemical structure was presumed from the result of on-column acetylation of kessoglycol.

were steam distilled and the distillates were extracted with benzene. The benzene solutions which contain valerian oil, were dried, evaporated to dryness and then dissolved in a small amount of acetone and run on the gas chromatograph.

Collection dates and oil contents of the valerian plants are shown in Table I.

#### Gas chromatography

Two kinds of packed columns, one with 1.5% SE-30 on Chromosorb W (60–80 mesh) (A) and the other with 12% DEGS on Gas Chrom P (80–100 mesh) (B), were used in this work. For the former packing a stainless steel column (2.25 m × 4 mm) was installed in a Shimadzu Model GC-1B and for the latter a glass one (1.87 m × 4 mm) in a Shimadzu Model GC-1C. Both types of column were equipped with a hydrogen flame ionization detector.

One to two  $\mu$ l of the acetone solution of the standard samples and valerian oils were used for injection into the gas chromatograph.

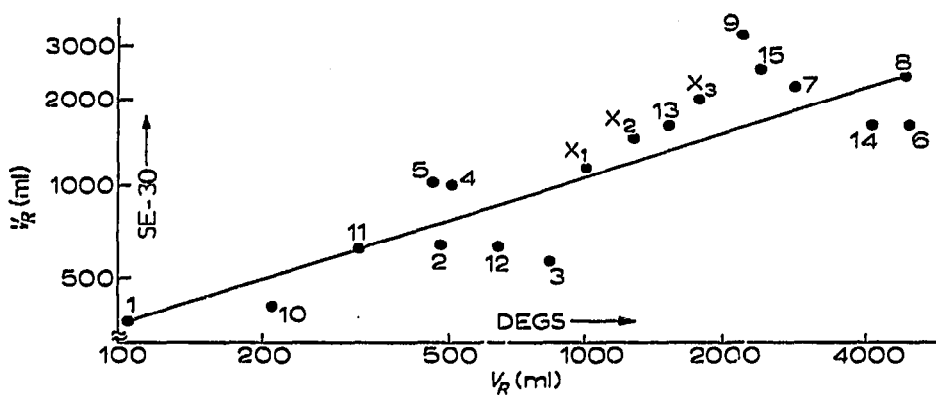


Fig. 1. Relationship between retention volumes and structures. For designations, see Table II and for  $X_1$ – $X_3$ , see p. 346.

#### On-column acetylation<sup>0</sup>

One  $\mu$ l of 1% solution of kessoglycol in acetone was first injected into the gas chromatograph, and followed as soon as possible by 3  $\mu$ l of acetic anhydride. Thus acetylation instantly proceeded on the column.

#### RESULTS AND DISCUSSION

There are two groups of kessane and valeranane skeletons in the valerian sesquiterpenoids. Most valerian sesquiterpenoids gave single sharp peaks which do not appear to decompose. Retention times and retention volumes of valerian sesquiterpenoids are shown in Table II.

The kessane group generally has hydroxyl and acetoxy substituents in the 2 and 8 positions, or both. The increase in retention times with increasing number of hydroxyl groups is especially notable. Kessane itself, which has no hydroxyl group, has the lowest retention time, while  $\alpha$ -kessyl alcohol and kessanol having 2- and 8-hydroxyl groups, respectively, have a medium retention time, and kessoglycol having two hydroxyl groups is retained the longest. The retention time of  $\alpha$ -kessyl alcohol is shorter than that of kessanol, because  $\alpha$ -kessyl alcohol has intermolecular

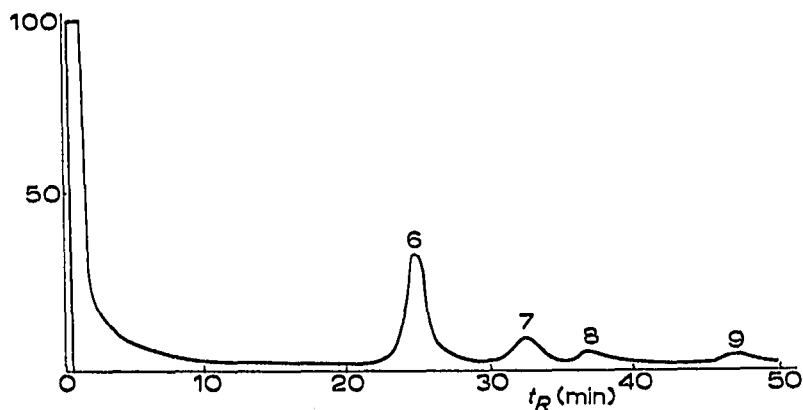


Fig. 2. Gas chromatogram resulting from on-column acetylation of kessoglycol. 6 = Kessoglycol; 7 = 8-acetoxykessan-2-ol; 8 = 2-acetoxykessan-8-ol; 9 = kessoglycol diacetate. Conditions: Shimadzu Model GC-1C, U-shaped glass column (1.87 m  $\times$  4 mm) packed with 1.5% SE-30 on Chromosorb W (60–80 mesh).  $N_2$  flow rate: 69.8 ml/min. Column temp. 158°; detector temp. 200°; flash heater temp. 250°.

hydrogen bonding<sup>10</sup> in its chemical structure, whereas there is no hydrogen bonding in kessanol.

Acetyl derivatives of the valerian sesquiterpenoids showed longer retention times than the corresponding hydroxyl compounds on the SE-30 column, especially in the case of acetylated compounds with a 2-hydroxyl group, where it was much longer. This fact is probably due to the disappearance of hydrogen bonding. However, the opposite was observed on using a DEGS column as polyester type stationary liquid phase; for example the correlations of retention times between kessyl acetate and kessanyl acetate, 8-acetoxykessan-2-ol and 2-acetoxykessan-8-ol; kanokonol and kanokonol acetate are also worthy of note.

Employing two kinds of stationary liquid phases such as SE-30 and DEGS, logarithms of retention volumes of valerian sesquiterpenoids were plotted in the graph illustrated in Fig. 1. By joining the three points due to kessane, valeranone and 2-acetoxykessan-8-ol, a straight line was obtained. Acetyl derivatives were plotted in the upper part of the line, while hydroxyl derivatives were plotted in the lower part. Some sesquiterpenoids having no hydroxyl and no acetoxyl groups or an equal number of non-bonding hydroxyl and acetoxyl groups were recorded on the line. Thus

TABLE III

THE RELATIVE AMOUNTS OF SESQUITERPENOIDS IN SOME JAPANESE VALERIAN ROOTS

Compounds	$t_R$	Samples				
		A	B	C	D	E
I	5.8	++++	+	+	+	
	6.2	+				
10	6.6		+	+	+	+
	6.9	++	+	+	+	+
	7.4	+	+	+	+	
	8.6		+	+	+	
	9.2	+	+	+	+	+
11	9.8	+	+	+	+	+
	10.7	++	+	+	+	
3	11.8				+	+
	12.5	+	+	+	+	
	13.1		+	+		
4	14.3	++	+	+	+	+
	16.8					+
	17.0	++++	+++	+++	+++	
	17.8	?	?	?	+	
X <sub>1</sub>	19.9	++	+	+	+	+
	21.7				+	
X <sub>2</sub>	22.9		+	+		+
	25.3	+++	++	++	++	++
14	28.3	+	+	+	+	
X <sub>3</sub>	32.4	++++	+++	+++	+++	+++
	35.5	+				
15	42.3		++	++	++	
	47.6					+
9	53.7	++++	++++	++	++++	
	60.5					+

A packed column with 1.5% SE-30 on Chromosorb W was used for this analysis. Valeranone = 1; +  $\leq$  1; 1 < ++  $\leq$  5; 5 < +++  $\leq$  10; 10 < ++++.

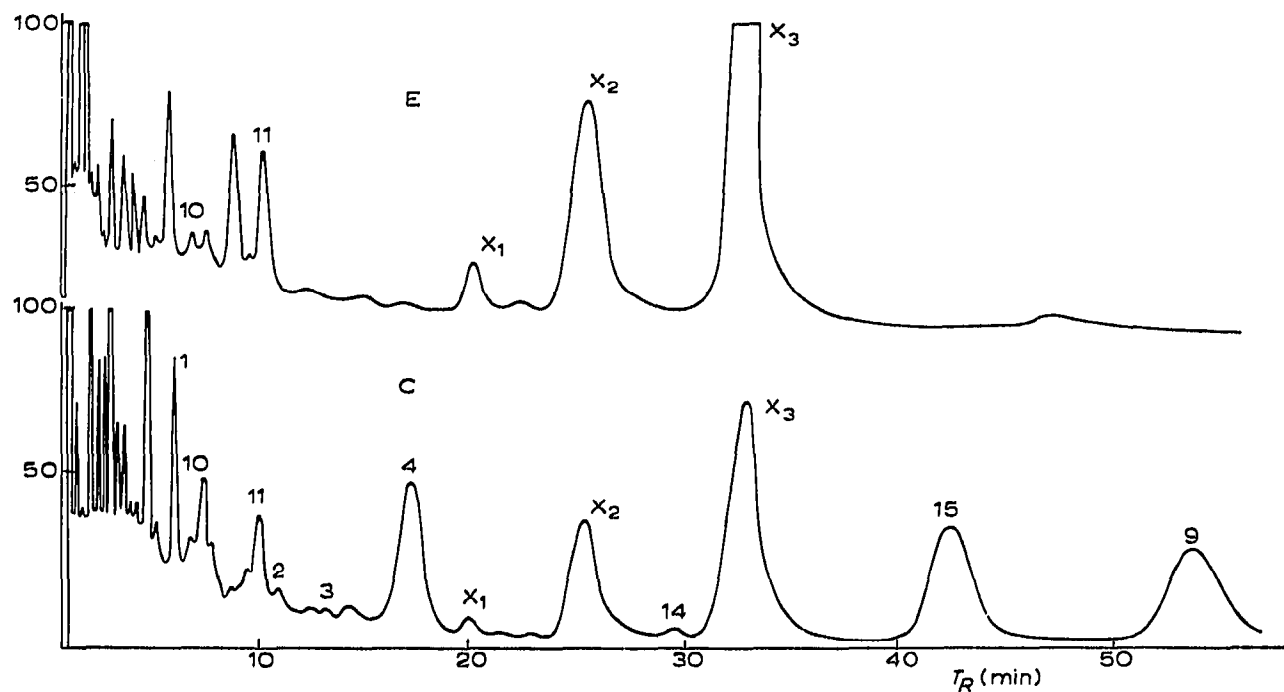


Fig. 3. Gas chromatograms of samples C and E with 1.5% SE-30. Conditions the same as in Table II. For designations, see Table II and for  $X_1$ - $X_3$ , see p. 346.

two or three unknown substances,  $X_1$ ,  $X_2$  and  $X_3$ , which we first found gas-chromatographically, are possibly acetyl derivatives.

From this point of view gas-liquid chromatography should provide a powerful tool for the identification and structural study of valerian sesquiterpenoids.

On-column acetylation of kessoglycol resulted in acetylation of hydroxyl groups to give sharp peaks with little tailing, as shown in Fig. 2.

The peaks obtained were identical with those of authentic samples except for 8-acetoxkykessan-2-ol, and are very effective for the analysis of valerian oil gas chromatograms.

The results of the gas chromatographic analysis of valerian oils are shown in Table III and some gas chromatograms in Figs. 3 and 4.

Gas chromatograms of samples A, B, C and D were very similar, except for the absence of maaliol and kanokonyl acetate in A. On the other hand, the gas chromatogram of E was distinctly different from that of the other oils. It appeared that sesquiterpenoids having a kessane nucleus are absent or only present in small amounts in E. Large amounts of kessoglycol diacetate and kessyl acetate were found in A, B, C and D but not in E. Considerable amounts of  $X_1$ ,  $X_2$ , and  $X_3$  and small amounts of valeranone, a new hypotensive and tranquilizing agent, were observed in all the valerian oils.

TAKEMOTO and his co-workers have already reported on the constituents of some Japanese<sup>11-13</sup> and European<sup>14</sup> valerian roots. We have also estimated the amounts of valerian sesquiterpenoids in five kinds of Japanese valerian oils using gas-liquid chromatography and got almost the same results in B and D as those in TAKEMOTO's paper<sup>12</sup>, except for kessanol which they found in D only, but we in both B and D.

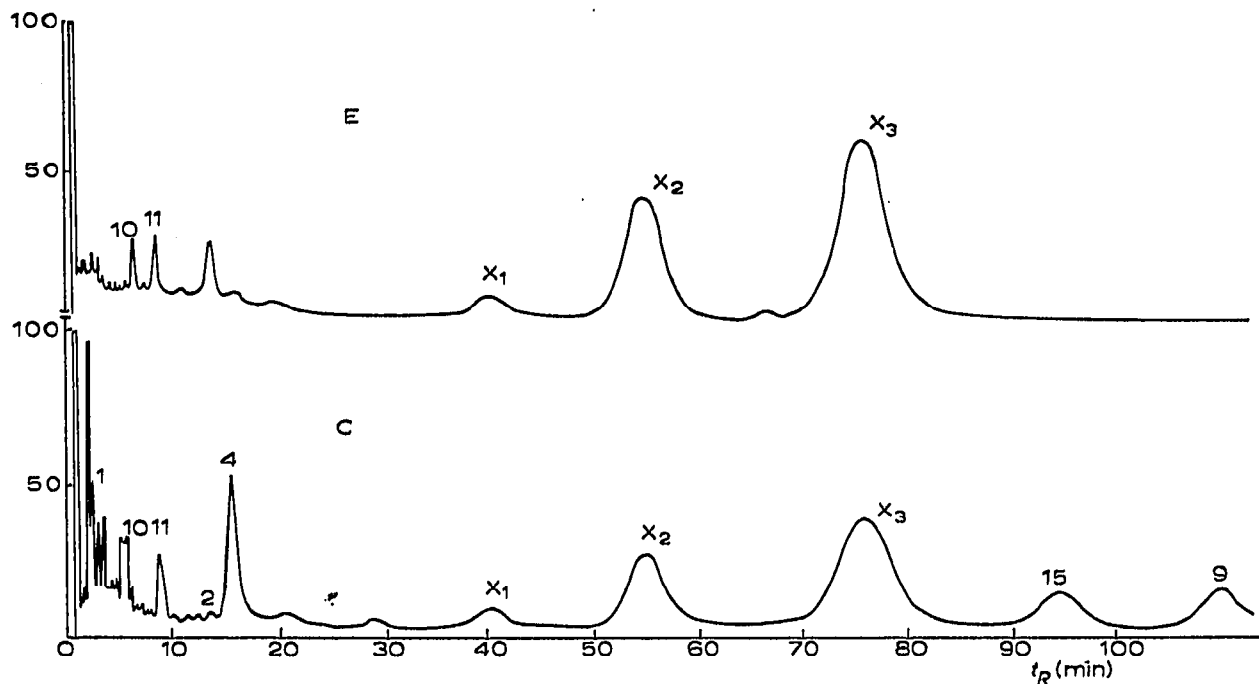


Fig. 4. Gas chromatograms of samples C and E with 12% DEGS. Conditions: A Shimadzu Model GC-1B, stainless steel column (2.25 m  $\times$  4 mm) packed with 12% DEGS on Gas Chrom P (80-100 mesh).  $N_2$  flow rate: 75.5 ml/min. Column temperature: 152°; detector temperature: 210°; flash heater temperature: 240°. For designations, see Table II and for  $X_1$ - $X_3$ , see p. 346.

From these results, morphological character and chromosome numbers<sup>15</sup>, it seems likely that the original valerian plants are closely related species and fairly distant from that of E.

#### ACKNOWLEDGEMENTS

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#### SUMMARY

The gas chromatographic behavior of fifteen kinds of valerian sesquiterpenoids was investigated using SE-30 and DEGS as stationary liquid phases.

The relationship between the presence of hydrogen bonding in the chemical structure and its effect on the retention time is discussed.

The present method will distinguish between various valerian oils and be useful for chemotaxonomical research into valerianaceous plants.

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